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Supplemental Information

TRIM9-Mediated Resolution of Neuroinflammation Confers Neuroprotection upon Ischemic Stroke in Mice

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Supplemental Figures

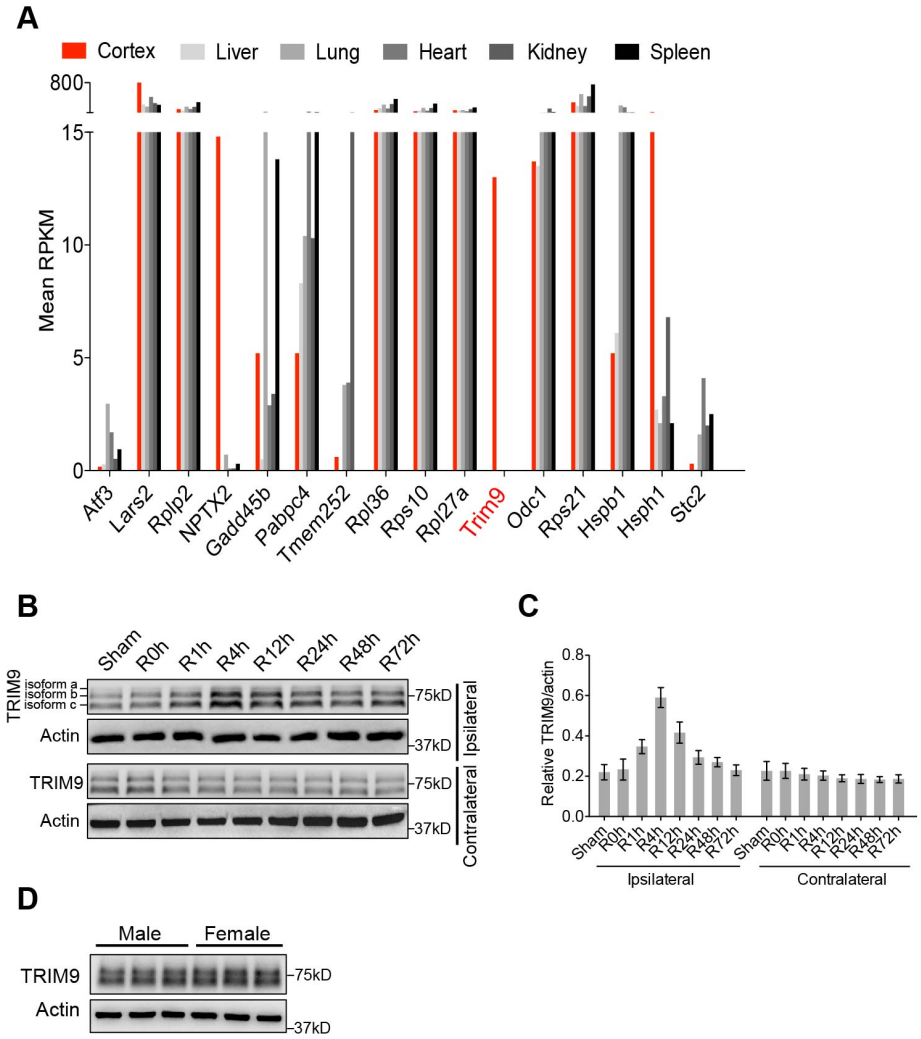

Figure S1. TRIM9 upregulation in mouse brains after stroke. Related to Figure 1. (A) Expression profiles of the top20 upregulated genes in the indicated mouse organs based on the published RNAseq data which are currently available from NCBI (see Methods). **(**B and C**)** Representative immunoblot (B) and quantification (C) of TRIM9 protein in ischemic brain tissues from C57BL/6J mice at the indicated time-points after 30min MCAO. The antibody used here reacted with three isoforms a/b/c of TRIM9 and the isoform b was used to quantify. *n*=3 mice per group. Data are shown as mean ± s.d. (D) Immunoblots of TRIM9 protein in brain tissues from three 12-week-old C57BL/6J male or female mice without stroke.

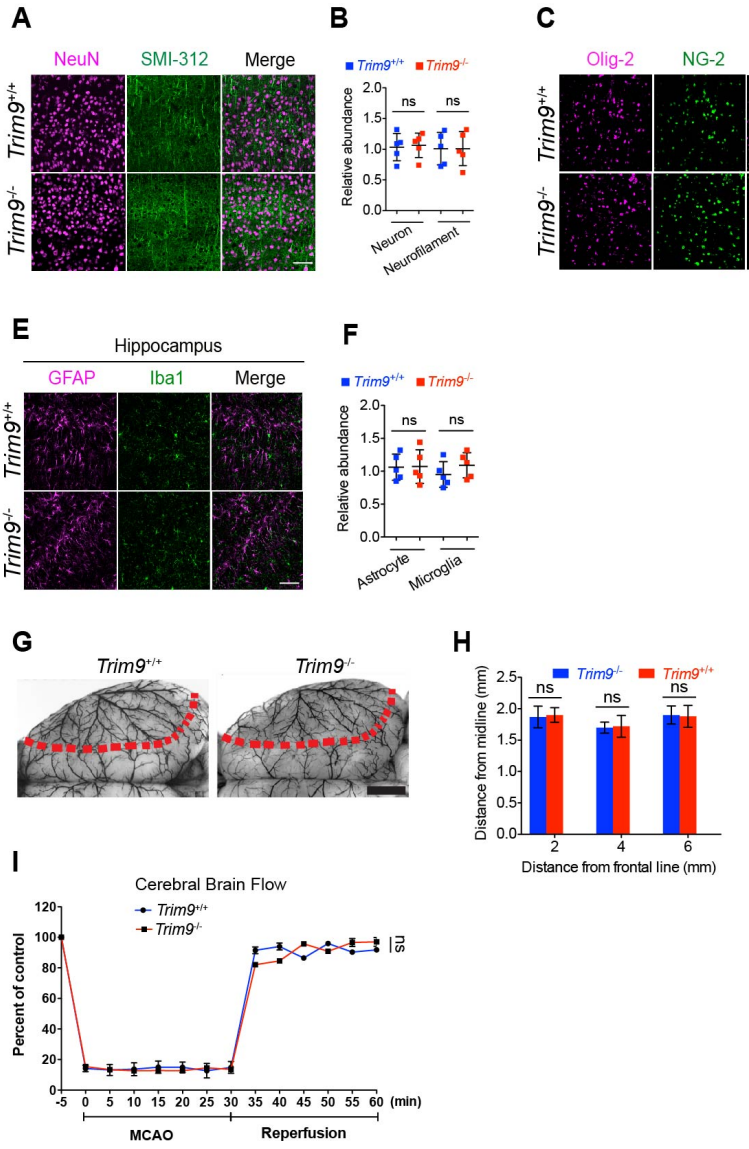

Figure S2. Characterization of *Trim9*$^{-/-}$ mice. Related to Figure 2. Immunostaining and quantification of neuronal and glial markers on brain sections of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice: A and B for neuron (NeuN^{+}) and neurofilament (SMI-312^{+}); C and D for oligodendrocytes (Olig2^{+}) and oligodendrocyte precursor cell (OPC, Olig2^{+} and NG-2^{+} double positive); and E and F for astrocytes (GFAP^{+}) and microglia (Iba1^{+}) in hippocampus. ns (non-significant) by Student's *t*-test. Scale bar, 30μm. *n*=5 mice per group. (G and H) The gross anatomy of the MCA territory of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice as shown by vascular infusion of latex mixed with carbon black (see methods for details). The distance from the midline of the anastomotic line between the MCA and anterior cerebral artery (ACA) vascular territory was determined from the frontal line. ns by Student's *t*-test. Scale bar, 2 mm. (I) Time course of CBF by laser doppler (LD) at 5 min intervals before, during and after 30 min of MCAO in *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice. ns by two-way ANOVA. *n*=3 mice per group. Data in B, D, F, H and I are shown as the mean ± s.d.

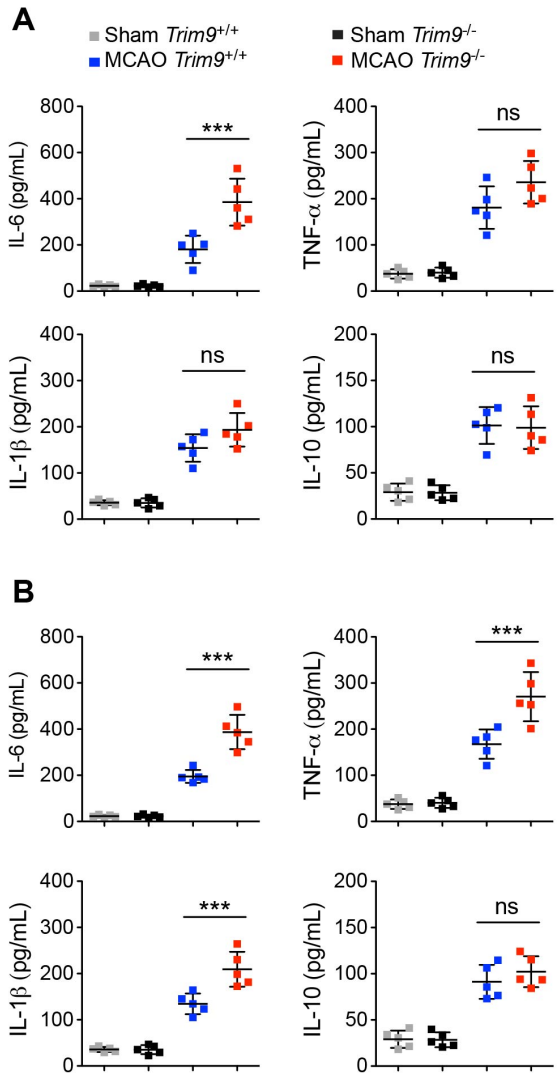

Figure S3. ELISA analysis of inflammatory mediators in ischemic mouse brains of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice. Related to Figure 2. ELISA analysis of IL-6, TNF-α, IL-1β, and IL-10 in hemispheric brain tissues of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice treated with 30 min MCAO and R24h (A) or R36h (B). *n*=5 mice per group. ***P<0.001 by one-way ANOVA followed by Bonferroni's *post hoc* analysis. ns, non-significant. Data are shown as mean ± s.d.

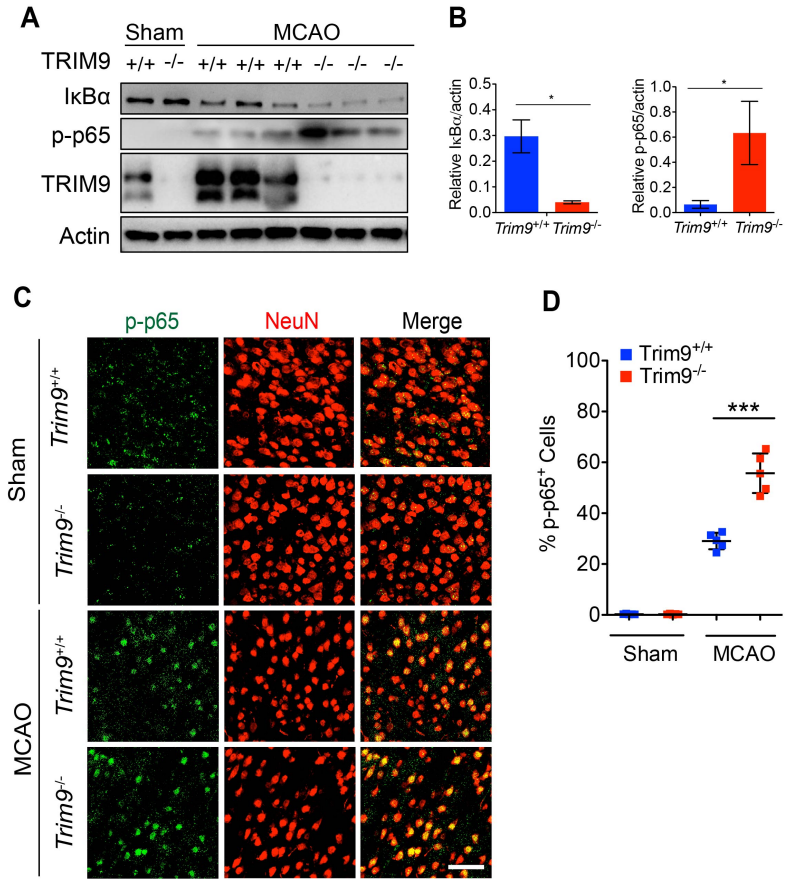

Figure S4. NF-κB signaling activity in ischemic brains of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice. Related to Figure 4. (A) Immunoblot analysis of IκBα degradation in ipsilateral hemisphere of brain tissues from *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice 24h after 30 min MCAO. *n*=3 mice upon MCAO. (B) Quantification of IκBα and p-p65 relative to actin after MCAO from A. **P*<0.05 by Student's t test. (C and D) Immunostaining (C) and quantification (D) of p-p65^{+}NeuN^{+} cells in the peri-infarct regions of ischemic brain from*Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ mice at 24 h after 30min MCAO. Scale bar, 30μm. *n*=5 mice per group. ****P*<0.001 by Student's *t*-test. Data in B and D are shown as mean ± s.d.

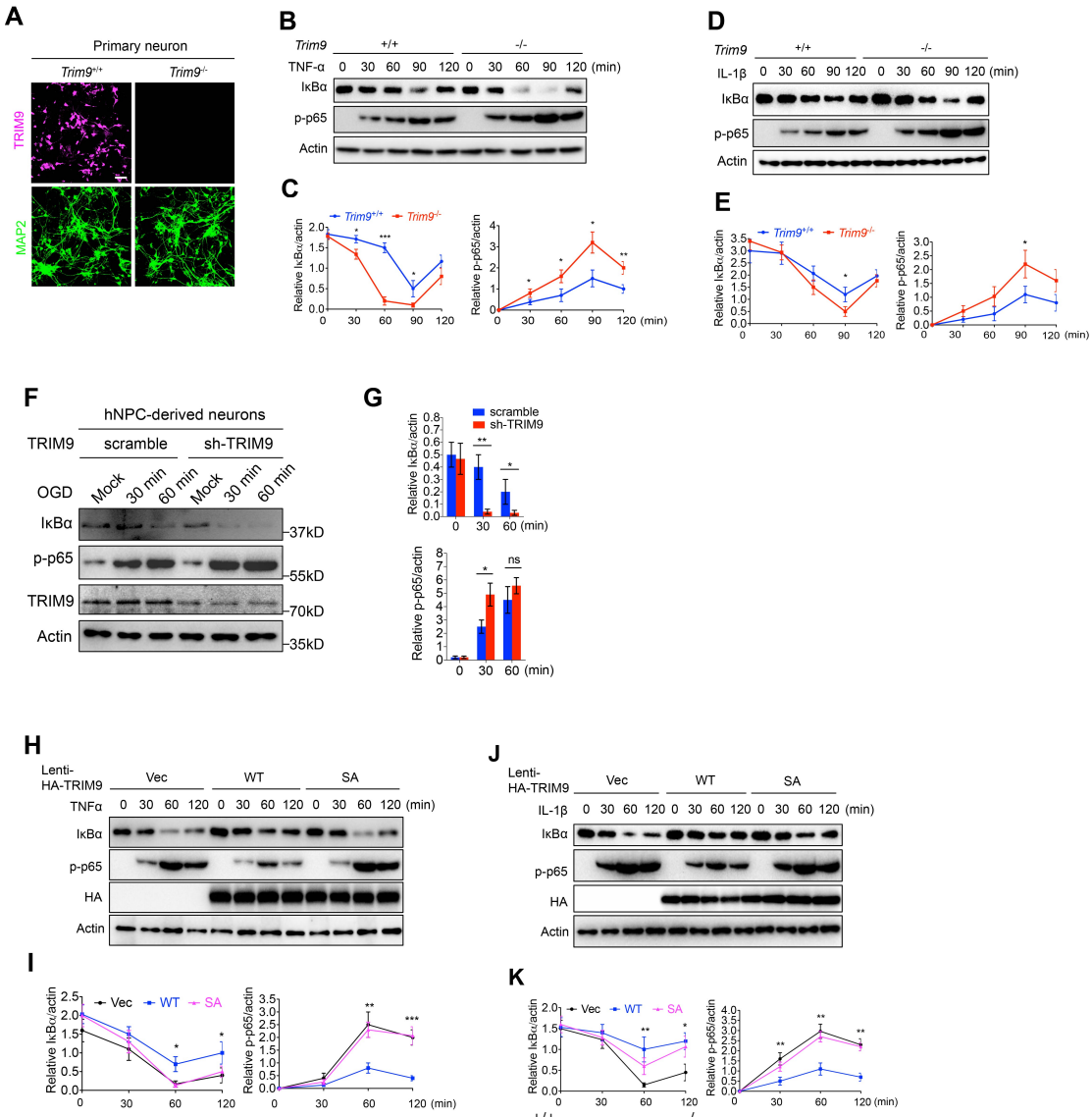

Figure S5. NF-κB signaling activity in *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ neurons upon stimulation. Related to Figure 4. (A) Immunostaining of TRIM9 and MAP2 in the cultured primary cortical neurons of *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ embryos. Scale bar, 30μm. (B-E) Immunoblot analysis (B and D) and quantification (C and E) of IκBα and p-p65 in *Trim9*$^{+/+}$ and *Trim9*$^{-/-}$ primary neurons upon TNF-α (25ng/ml) or IL-1β (25ng/ml) stimulation. **P*<0.05 and ****P*<0.001 by Student's *t*-test. (F and G) Representative immunoblot (F) and quantification (G) of IκBα and p-p65 in hNPC-derived neurons infected with scramble- or TRIM9-specific shRNA lentivirus at 24h after 30 or 60min of OGD treatment. **P*<0.05 and ***P*<0.01 by Student's t test. ns, non-significant. (H-K) Immunoblot analysis (H and J) and quantification (I and K) of IκBα and p-p65 in *Trim9*$^{-/-}$ primary neurons infected with Vec, TRIM9 (WT) or TRIM9 SA mutant lentivirus, followed by TNF-α (25ng/ml) or IL-1β (25ng/ml) stimulation. **P*<0.05, ***P*<0.01 and ****P*<0.001 by Student's *t*-test. Data in C, E, G, I and K are shown as mean ± s.d.

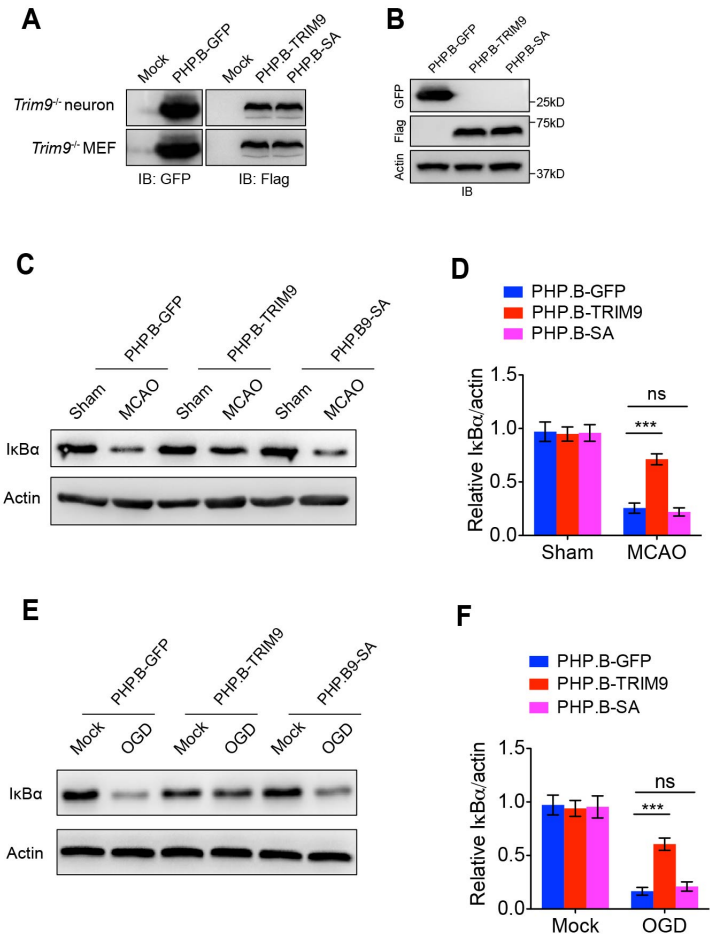

Figure S6. AAV-PHP.B-mediated expression of TRIM9, not GFP and SA mutant, suppresses NF-κB signaling and inflammatory cytokines production in ischemic mouse brains or primary neurons. Related to Figure 5. (A) Representative immunoblots of GFP and Flag in *Trim9*$^{-/-}$ primary neurons and mouse embryo fibroblasts (MEFs) infected by the indicated AAV. (B) Representative immunoblots of GFP, Flag-TRIM9 and actin in the brain tissues from *Trim9*$^{-/-}$ mice injected with the indicated AAV. At 21d post-infection, brain tissue extracts were used for IB with indicated antibodies. (C and D) Representative immunoblot (C) and quantification (D) of IκBα in tissue extracts from ischemic brain hemispheres of *Trim9*$^{-/-}$ mice received indicated AAV vectors at 24h after 30min MCAO. n=5 mice per group. ***P<0.001 by Student's t-test. ns, non-significant. (E and F) Representative immunoblot (E) and quantification (F) of IκBα level in *Trim9*$^{-/-}$ primary neurons infected with indicated AAV vectors at 24h after 30min OGD treatment. ***P<0.001 by Student's t-test. ns, non-significant. Data in D and F are shown as mean ± s.d.

Table S1: The top 20 statistically upregulated genes from RNA-seq. Related to Figure 1.

Order	Genes
1	Hspa1b
2	Hspa1a
3	Atf3
4	Lars2
5	Mir6236
6	Rplp2
7	Nptx2
8	Gadd45b
9	Pabpc4
10	Tmem252
11	Rpl36
12	Cebpb
13	Rps10
14	Rpl27a
15	Trim9
16	Odc1
17	Rps21
18	Hspb1
19	Hsph1
20	Stc2

Table S2: The genes from the pathway of neurological disease and inflammation in RNA-seq. Related to Figure 1.

No.	Genes	No.	Genes
1	Ch25h	42	Pebp1
2	Socs3	43	Gadd45b
3	Myh9	44	Icam1
4	Trim9	45	Zfp36
5	Hspa1b	46	Bdbf
6	Ddc	47	Gadd45g
7	Flt3	48	Klf10
8	Hspa1b/1a	49	Hmgn1
9	Serpina3	50	Cebpd
10	Mt2	51	Hmgb2
11	Hba1/2	52	Myh11
12	Ddb2	53	Tubb2b
13	Hspa5	54	C11orf96
14	Eef1g	55	Tgm2
15	Grasp	56	Jun
16	Tmsb4x	57	Ccl2
17	Nptx2	58	Hexb
18	Ttr	59	Dnajb1
19	Adamts1	60	Ifrd1
20	Pdyn	61	Ier5
21	Egr4	62	Arc
22	Etnppl	63	Mt-nd6
23	Lmna	64	Rgs2
24	Ier3	65	Relb
25	Junb	66	Egr1
26	Scn10a	67	Plaur
27	Hmga1	68	Cebpb
28	Maff	69	Sertad1
29	Pdgfb	70	Hbb
30	Inhba	71	Fos
31	Hspa8	72	Ftl
32	H3f3a/b	73	Sstr2
33	Ccl4	74	Hs3st2
34	Acta2	75	Lingo1
35	Npas4	76	Tagln
36	Pdlim1	77	Ptgs2
37	Cd14	78	Wfs1
38	Alas2	79	Ldha
39	Cyr61	80	Hmgcr
40	Hspb1	81	Ubc
41	Apod		

Table S3: Primers used in RT-qPCR. Related to Figure 2.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Il-6	CAAAGCCAGAGTCCTTCAGAG	GTCCTTAGCCACTCCTTCTG
Il1β	AAGGGCTGCTTCCAAACCTTTGAC	ATACTGCCTGCCTGAAGCTCTTGT
Tnfa	CTTCTGTCTACTGAACTTCGGG	TGATCTGAGTGTGAGGGTCTG
Ccl11	CAGATGCACCCTGAAAGCCATA	TGCTTTGTGGCATCCTGGAC
Ccl3	CACCACTGCCCTTGCTGTT	AGGAGAAGCAGCAGGCAGTC
Ccl4	GCTCCAAGCCAGCTGTGGTA	CGCTGGAGCTGCTCAGTTC
Ccl5	TGCCCACGTCAAGGAGTATTT	TCTCTGGGTTGGCACACACTT
Cxcl10	TGCTGGGTCTGAGTGGGACT	CCCTATGGCCCTCATTCTCAC
Ccl2	CATCCACGTGTTGGCTCA	GATCATCTTGCTGGTGAATGAGT
Ccl9	CCCTCTCCTTCCTCATTCTTACA	AGTCTTGAAAGCCCATGTGAAA